Research Article

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Synthesis of three isotopically labeled versions and a metabolite of Aurora A kinase inhibitor

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Sodium ring-[^{14}C]-4-[[9-chloro-7-(2,6-difluorophenyl)-*5H*-pyrimido[5,4-*d*][2]benzazepin-2-yl]amino]-benzoate (1A, MLN8054), an Aurora A kinase inhibitor, was synthesized from [^{14}C]-cyanamide in two steps in an overall radiochemical yield of 7%. The intermediate, [^{14}C]-4-guanidinobenzoic acid, was prepared by coupling [^{14}C]-cyanamide with 4-aminobenzoic acid. Sodium carboxyl-[^{14}C]-4-[[9-chloro-7-(2,6-difluorophenyl)-*5H*-pyrimido[5,4-*d*][2]benzazepin-2-yl]amino]-benzoate (1B) was synthesized from carboxyl-[^{14}C]-4-guanidinobenzoic acid in one step in a radiochemical yield of 35%. [D_4,^{15}N]-4-[[9-chloro-7-(2,6-difluorophenyl)-*5H*-pyrimido[5,4-*d*][2]benzazepin-2-yl]amino]-benzoic acid (1C) was synthesized from [$^{15}N_2$]-cyanamide and [D_4]-4-aminobenzoic acid in two steps in an overall yield of 37%. The major metabolite, β-acyl glucuronide of 4-[[9-chloro-7-(2,6-difluorophenyl)-*5H*-pyrimido[5,4-*d*][2]benzazepin-2-yl]amino]-benzoic acid (14), was synthesized from *D*-glucuronic acid in three steps in an overall yield of 1%. The key intermediate for synthesis of glucuronide was prepared by HATU catalyzed coupling of 4-[[9-chloro-7-(2,6-difluorophenyl)-*5H*-pyrimido[5,4-d][2]benzazepin-2-yl]amino]-benzoic acid with allyl glucuronate.

Keywords: sodium ring-[^{14}C]-4-[[9-chloro-7-(2,6-difluorophenyl)-*5H*-pyrimido[5,4-*d*][2]benzazepin-2-yl]amino]-benzoate; sodium carboxyl-[^{14}C]-4-[[9-chloro-7-(2,6-difluorophenyl)-*5H*-pyrimido[5,4-*d*][2]benzazepin-2-yl]amino]-benzoate; [D_4, ^{15}N]-4-[[9-chloro-7-(2,6-difluorophenyl)-*5H*-pyrimido[5,4-*d*][2]benzazepin-2-yl]amino]-benzoic acid; β-acyl glucuronide of 4-[[9-chloro-7-(2,6-difluorophenyl)-*5H*-pyrimido[5,4-d][2]benzazepin-2-yl]amino]-benzoic acid; [^{14}C]-4-guanidinobenzoic acid; [D_4, ^{15}N]-4-guanidinobenzoic acid

Introduction

Aurora A kinase plays an essential role in the proper assembly and function of the mitotic spindle.[1,2] Inhibition of Aurora A kinase represents an attractive modality for therapeutic intervention of human cancers.[3] The biological activity of the recently discovered Aurora A inhibitor, 4-[[9-chloro-7-(2,6-difluorophenyl)-*5H*-pyrimido[5,4-*d*][2]benzazepin-2-yl]amino]-benzoic acid (1, Figure 1),[1–4] led to detailed investigations on its disposition characteristics. The radiolabeled versions were required to assist such investigations, especially in metabolite profiling and whole body autoradiography studies in experimental animals. The stable isotope labeled version was also required as an internal standard for mass spectrometry-based bio-analytical assays. The major metabolite, β-acyl glucuronide of 4-[[9-chloro-7-(2,6-difluorophenyl)-*5H*-pyrimido[5,4-d][2]benzazepin-2-yl]amino]-benzoic acid, was required to support the studies of inhibitory effects on transporters (PGP, BCRP, etc.) and as a reference standard.[5]

Results and discussion

The structure of 4-[[9-chloro-7-(2,6-difluorophenyl)-*5H*-pyrimido[5,4-*d*][2]benzazepin-2-yl]amino]-benzoic acid (1) provides two potential sites for labeling, either on the top pyrimidinimine benzoic acid portion or the bottom benzoazepine portion of the molecule (Scheme 1).[4] Preclinical metabolism results indicated that radiolabeling either portion of the molecule would be adequate for the intended ADME studies in animals. For expedient synthesis, we chose labeling guanidinobenzoic acid 2 instead of benzoazepine 7 for both stable isotope and radiolabeled versions (Scheme 1).

There are two possible approaches to radiolabel compound 2, either on the gualidine portion to prepare the ring-labeled 1 or on carboxylic group to the carboxyl-labeled 1 (Scheme 1). We first chose to synthesize the ring-labeled compound 1 either from [^{14}C]-thiourea or [^{14}C]-cyanamide. In the unlabeled synthesis, the yield of aminoiminomethanesulfonic acid 4 from 5 and ethaneperoxoic acid was only 14%.[6] But the reaction of cyanamide 6 and 3 resulted in a reasonable yield.[7] Therefore [^{14}C]-cyanamide 6A was chosen the radiolabeled precursor (Scheme 2). Reaction of 6A with 4-aminobenzoic acid 3 provided [^{14}C]-4-guanidinobenzoic acid

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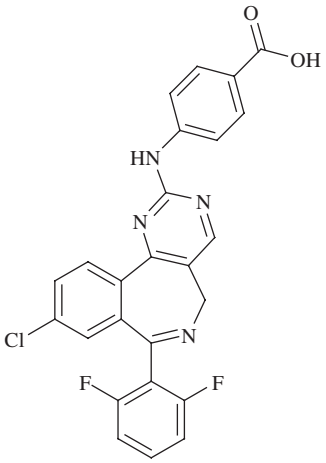
2A in a yield of 43%. Ring-[^{14}C]-4-[[9-chloro-7-(2,6-difluorophenyl)-*5H*-pyrimido[5,4-*d*][2]benzazepin-2-yl]amino]-benzoate (1A) was prepared by K_2CO_3-mediated coupling of 2A with 7 in methanol (16%).[4]

The solid C-14-labeled compound 1A was found to be unstable, but its solution of ethanol/water (or methanol/water) was stable. For a better stability in solid phase, carboxyl-[^{14}C]-4-[[9-chloro-7-(2,6-difluorophenyl)-*5H*-pyrimido[5,4-*d*][2]benzazepin-2-yl]amino]-benzoate (1B) was synthesized from commercially available carboxyl-[^{14}C]-4-guanidinobenzoic acid in a yield of 35% (Scheme 2). The solid compound 1B was found to be much more stable than the solid compound 1A.

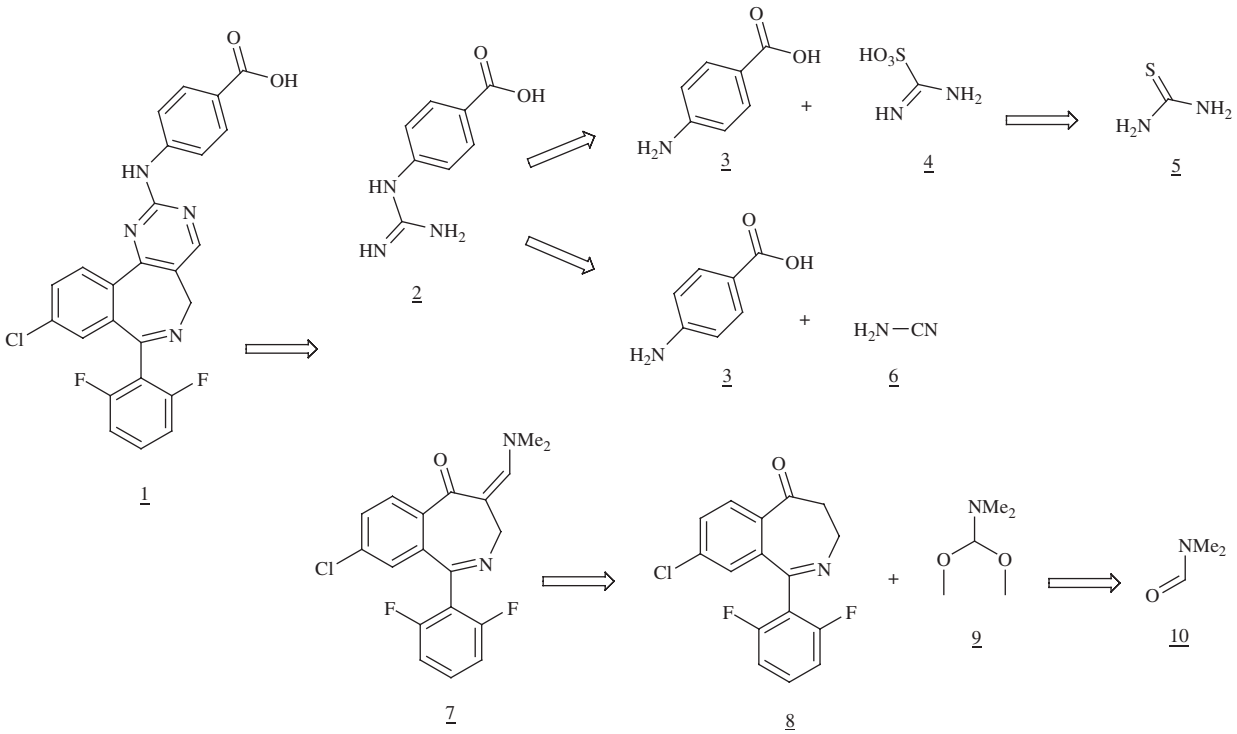


1

Figure 1. The structure of MLN8054.

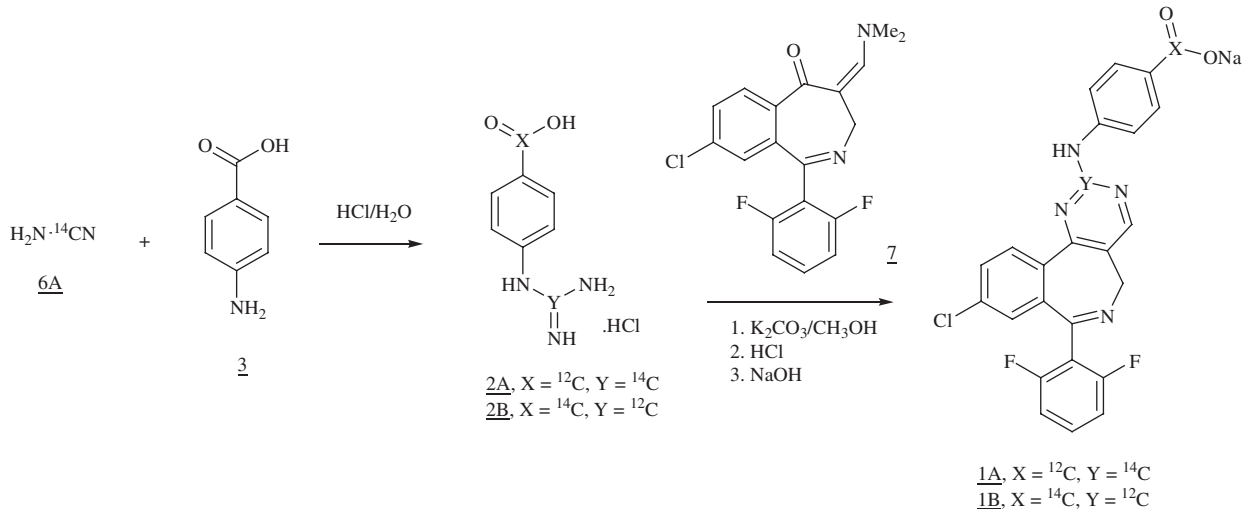
Because 1 contains a chlorine atom, a labeled version that is 5 amu higher than the unlabeled version is required to ensure complete separation of labeled and unlabeled molecular ion clusters during mass spectrometric assays of 1. To increase the molecular weight, labeling of the pyrimidinimine benzoic acid portion of the molecule or benzoazepine portion was required. Labeling the 4-guanidinobenzoic acid 2 with deuterium and nitrogen-15 was chosen. Similar to compound 1A, compound 1C was synthesized from [^{15}N]-cyanamide 6B and [D_4]-4-amino-benzoic acid 3A (Scheme 3).

To chemically synthesize acyl glucuronides, several strategies have been utilized. Mesmaeker *et al*. employed an approach of a fully protected glucuronic acid that included ten-step synthesis with several protections and deprotections.[8] Juteau *et al*. utilized a Mitsunobu reaction of allyl glucuronate with carboxylic acids.[9] But the ratio of β/α was only from 5/1 to 2/1.[10] Stachulski *et al*. reported a more selective method for the synthesis of this type of compound by β-acylation of allyl glucuronate with carboxylic acids catalyzed by HATU (*o*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate).[10] Therefore, Stachulski's method was chosen for the synthesis of β-acyl glucuronide 14, (Scheme 4). The protected allyl glucuronate 12 was prepared from *D*-glucuronic acid 11 and allyl bromide.[10] HATU-mediated coupling of 12 with 1 provided the key intermediate compound 13. But no product was found when only acetonitrile was used as the solvent.[10] To dissolve the highly insoluble compound 1, the co-solvent of acetonitrile and *N*-methylmorpholine was utilized for the coupling reaction. β-Acyl glucuronide of 4-[[9-chloro-7-(2,6-difluorophenyl)-*5H*-pyrimido[5,4-*d*][2]benzazepin-2-yl]amino]-benzoic acid (14) was prepared by de-protection of 13 using $Pd(PPh_3)_4$ in conjunction with pyrrolidine in THF.[10]

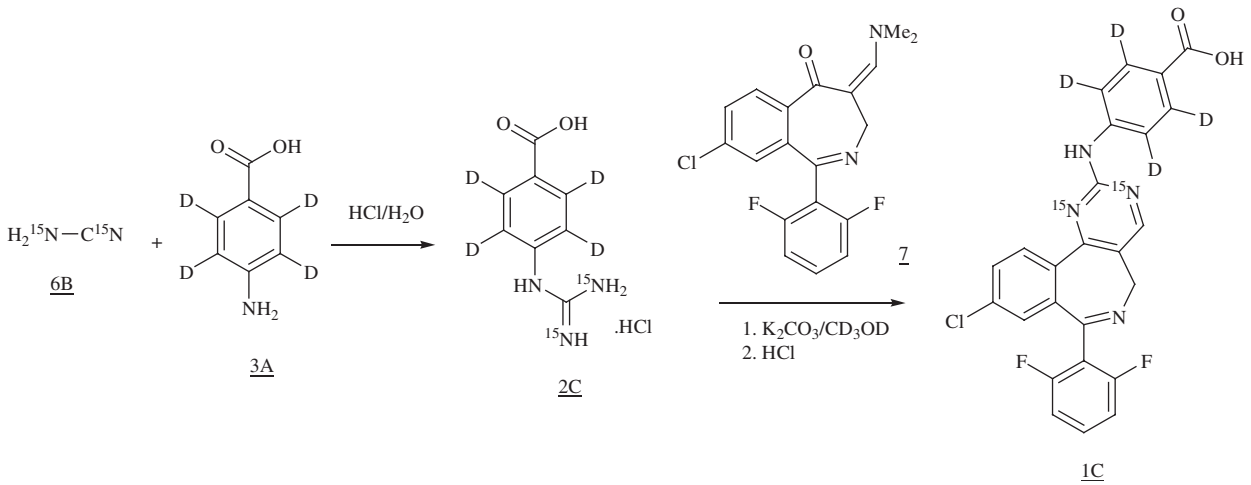


Scheme 1.

Journal of Labelled Compounds and Radiopharmaceuticals



Scheme 2.



Scheme 3.

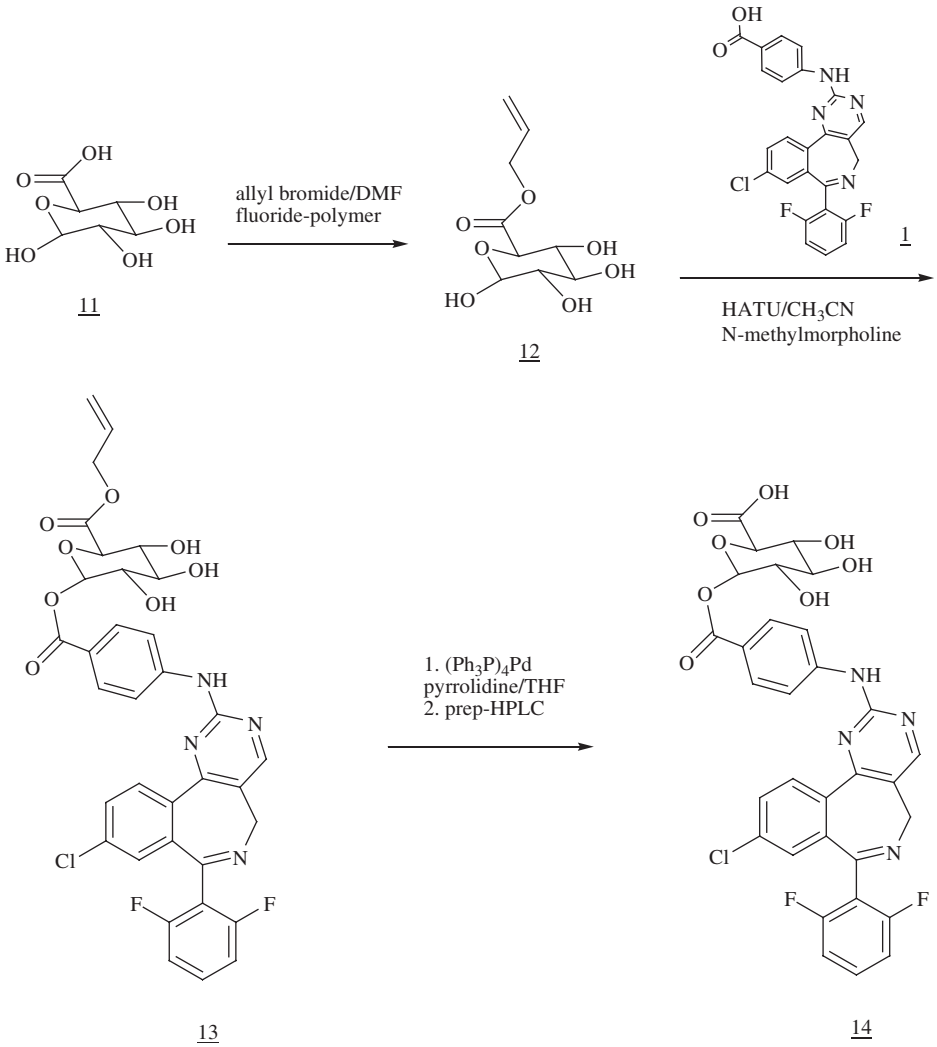
Experimental

General

All commercial reagents were used as supplied unless otherwise noted. [^{14}C]-Cyanamide was purchased from American Radiolabeled Chemicals, USA. Carboxyl-[^{14}C]-4-guanidinobenzoic acid was purchased from PerkinElmer Life Sciences, USA. [$^{15}N_2$]-Cyanamide was purchased from Aldrich, USA. [D_4]-4-Aminobenzoic acid was purchased from C/D/N Isotopes, Canada. Radioactivity was quantified by liquid scintillation counting using a Beckman LS6500 counter. Purities were determined by HPLC (Agilent 1100) on Luna C18(2) column (5 μm, 4.5 × 150 mm) eluted at 1 mL/min with A (0.1% formic acid in 99% water and 1% CH_3CN) and B (0.1% formic acid in 5% water and 95% CH_3CN). Elution was 0% B for 5 min, 0–100% B over 15 min, and 100% B holding for 5 min. UV detection was at 254 nm. Radioactive detection was with an IN/US β-Ram RHPLC detector using an Ultima Flo M scintillant at 3 mL/min. NMR spectra were recorded on either a Varian 600 or a 300 MHz spectrometer. LC-MS analyses were performed on an Agilent 1100 Series LC/MSD and a Thermo-Finnigan LCQ mass spectrometers. Column chromatography was performed on silica gel (230−400 mesh) supplied by SiliCycle, Canada.

[^{14}C]-4-Guanidinobenzoic acid (2A)[7]

Aqueous [^{14}C]-cyanamide solution (50%, 108 mCi, 2.5 mmol, 43 mCi/mmol) was added to a mixture of concentrated HCl (37%, 0.15 mL, 1.82 mmol) and 4-aminobenzoic acid (312 mg, 2.27 mmol) at 85°C. Another portion of concentrated HCl (37%, 0.04 mL, 0.454 mmol) was added. After stirring for 4 h at 85°C, the mixture was allowed to cool to room temperature. The solvents were removed by evaporation. The resulting solid was purified by preparative HPLC on a Luna column (5 μm 250 × 21.2 mm) using a gradient of 0.1% HCOOH in water and 0.1% HCOOH in acetonitrile. Product containing fractions were combined and dried under vacuum to yield 46 mCi (43%, 43 mCi/mmol) product. HPLC: chemical purity 95% (UV at 254 nm); radiochemical purity, 96%. LC-MS/MS: *m/z* 182 (M+1), 180.



Scheme 4.

Sodium ring-[^{14}C]-4-[[9-chloro-7-(2,6-difluorophenyl)-5*H*-pyrimido[5,4-d][2]benzazepin-2-yl]amino]-benzoate (1A)[4]

[^{14}C]-4-Guanidinobenzoic acid (2A) (41 mCi, 0.942 mmol, 43 mCi/mmol) and compound 7 (309 mg, 0.856 mmol) were added to a dry flask. Methanol (3.2 mL) and K_2CO_3 (260 mg, 1.88 mmol) were added under nitrogen. The resulting mixture was heated at 60°C under nitrogen overnight. After cooling to room temperature, water (0.9 mL) and 5 M HCl (0.64 mL) were added. The resulting solid was collected by centrifuge. The impure product was purified by preparative HPLC on a Luna column (5 μm, 250 × 21.2 mm) using a gradient of 0.1% HCOOH in water and 0.1% HCOOH in acetonitrile. Product containing fractions was combined and dried under vacuum. 1 N NaOH (1 eq) was added to the resulting solid dissolved in methanol. The solvents were removed to give 6.6 mCi (43 mCi/mmol, 16%) of the title compound. HPLC: 99.0% (UV at 300 nm); radiochemical purity, 99.2%. LC-MS/MS: *m/z* 479 (M+1), 481. ^{1}H-NMR (CD_3OD): δ 8.58 (NH, s), 8.38 (1H, d), 7.95 (2H, d), 7.80 (4H, m), 7.50 (1H, m), 7.30 (1H, s), 7.05 (2H, t), 4.00 (2H, s).

Sodium carboxyl-[^{14}C]-4-[[9-chloro-7-(2,6-difluorophenyl)-5*H*-pyrimido[5,4-d][2]benzazepin-2-yl]amino]-benzoate (1B)[4]

Carboxyl-[^{14}C]-4-guanidinobenzoic acid (2B) (114 mCi, 2.0 mmol, 57 mCi/mmol) and compound 7 (656 mg, 1.82 mmol) were added to a dry flask. Methanol (6.8 mL) and K_2CO_3 (553 mg, 4.0 mmol) were added under nitrogen. The resulting mixture was heated at 60°C under nitrogen overnight. After cooling to room temperature, water (2 mL) and 5 M HCl (1.4 mL) were added. The resulting solid was collected by filtration, dissolved in methanol (80 mL) and mixed with 1 N NaOH (1 eq). The solvents were removed. The resulting solid was crystallized from ethanol and dried to give 39.9 mCi (57 mCi/mmol, 35%) of the title compound. HPLC: 99.0% (UV at 300 nm); radiochemical purity, 98.4%. LC-MS/MS: *m/z* 479 (M+1), 481. ^{1}H-NMR (CD_3OD): δ8.58 (NH, s), 8.38 (1H, d), 7.95 (2H, d), 7.78 (4H, m), 7.50 (1H, m), 7.28 (1H, s), 7.05 (2H, t), 3.90 (2H, s).

[D_4,^{15}N]-4-guanidinobenzoic acid (2C)[7]

Concentrated DCl in D_2O (35%, 0.14 mL, 1.82 mmol) was added to [D_4]-4-aminobenzoic acid (282 mg, 2.0 mmol). [$^{15}N_2$]-cyanamide solution in D_2O (50%, 3.0 mmol) was added into the reaction mixture at 85°C. A further concentrated DCl in D_2O (35%, 0.04 mL, 0.454 mmol) was added. After stirring for 4 h at 85°C, the mixture was allowed to cool to room temperature. The solvents were removed by evaporation. Acetonitrile was added to the resulting solid. The mixture was filtered and washed with acetonitrile and acetone. The resulting solid was dried under

vacuum to yield 380 mg (80%) product. HPLC: 96% (UV at 254 nm). LC-MS/MS: *m/z* 186 (M+1). ^{1}H-NMR ($(CD_3)_2SO$): δ13.00 (COOH, s), 10.43 (1H, s), 7.78 (4H, s).

[D_4,^{15}N]-4-[[9-chloro-7-(2,6-difluorophenyl)-5*H*-pyrimido[5,4-d][2]benzazepin-2-yl]amino]-benzoic acid (1C)[4]

[D_4,^{15}N]-4-guanidinobenzoic acid (2C) (346, 1.38 mmol) and compound 7 (454 mg, 1.26 mmol) were added to a dry flask. [D_4]-Methanol (4.7 mL) and K_2CO_3 (382 mg, 2.77 mmol) were added under nitrogen. The resulting mixture was heated at 60°C under nitrogen overnight. After cooling to room temperature, D_2O (1.4 mL) and 5 M DCl in D_2O (0.9 mL) were added. The mixture was filtered and washed with methanol and water. The resulting solid was crystallized from [D_4]-Methanol. The purified solid was dried to give 394 mg (58%) of the title compound. HPLC: 96% (UV at 254 nm). LC-MS/MS: *m/z* 483 (M+1), 485. ^{1}H-NMR (CD_3OD): δ12.50 (COOH, s), 10.28 (NH, s), 8.78 (1H, d), 8.30 (1H, d), 7.90 (1H, m), 7.58 (1H, m), 7.38 (1H, s), 7.18 (2H, m), 4.90 (1H, s), 3.90 (1H, s).

D-allyl glucuronate 12[10]

D-glucuronic acid 11 (4.0 g, 20.6 mmol) in DMF (40 mL) was added allyl bromide (2.0 mL, 23 mmol) and polymer-supported fluoride (8.5 g, 22 mmol). The resulting mixture was stirred at 52°C under nitrogen overnight. The reaction mixture was cooled to room temperature and filtered. The filtrate was evaporated. The resulting residue was purified by chromatography on silica gel (heptanes/dichloromethane/ethanol) to yield 1.64 g (34%) product. This product was used in the next step without further characterization.

β-Allyl glucuronide of 4-[[9-chloro-7-(2,6-difluorophenyl)-5*H*-pyrimido[5,4-d][2]benzazepin-2-yl]amino]-benzoic acid 13

4-[[9-Chloro-7-(2,6-difluorophenyl)-5*H*-pyrimido[5,4-d][2]benzazepin-2-yl]amino]-benzoic acid 1 (2.95 g, 6.18 mmol), D-allyl glucuronate 12 (1.448 g, 6.18 mmol), and HATU (2.82 g, 7.42 mmol) were stirred in acetonitrile (32 mL) with *N*-methylmorpholine (32 mL) under nitrogen for 2 h. The reaction was quenched by adding Amberlyst A-15 (H^+ form, 24 g). After evaporation, the residue was purified by chromatography on silica gel (dichloromethane/ethanol) to get the product (385 mg, 9%). HPLC: 96% (UV at 254 nm). LC-MS/MS: *m/z* 693 (M+1), 695. ^{1}H-NMR ($(CD_3)_2SO$): δ 10.40 (1H, s), 8.80 (1H, s), 8.38 (1H, d), 8.20 (4H, s), 7.90 (1H, d), 7.60 (1H, m), 7.38 (1H, s), 7.20 (2H, t), 5.92 (1H, m), 5.65 (1H, d), 5.50 (1H, m), 5.38 (1H, m), 5.12 (1H, d), 4.62 (1H, d), 4.02 (1H, d), 3.78 (1H, s), 3.40 (2H, m), 3.16 (1H, s).

β-Acyl glucuronide of 4-[[9-chloro-7-(2,6-difluorophenyl)-5*H*-pyrimido[5,4-d][2]benzazepin-2-yl]amino]-benzoic acid (14)

To a solution of compound 13 (83 mg, 0.12 mmol) in THF (2 mL) at 0°C was added $Pd(PPh_3)_4$ (14 mg, 0.12 mmol) followed by pyrrollidine (0.01 mL, 0.12 mmol). The reaction solution was stirred half hour at 0°C. After evaporation, the residue was purified by preparatory HPLC on a C18(2) Luna column (water/acetonitrile/0.1% HCOOH) to get the product (28 mg, 36%). HPLC: 99% (UV at 254 nm). LC-MS/MS: *m/z* 653 (M+1), 655. ^{1}H-NMR (15% CD_3CN/85% D_2O): δ10.39 (NH, s), 8.70 (1H, s), 8.20 (1H, d), 8.00 (2H, s), 7.80 (2H, m), 7.65 (1H, m), 7.45 (1H, m), 7.40 (1H, s), 7.00 (2H, s), 5.70 (1H, s), 4.78 (1H, s), 3.90 (1H, s), 3.82 (2H, d), 3.50 (2H, d).

Conclusion

In summary, practical methods for the preparation of carbon-14 and stable isotope-labeled Aurora A inhibitor, 4-[[9-chloro-7-(2,6-difluorophenyl)-5*H*-pyrimido[5,4-d][2]benzazepin-2-yl]amino]-benzoic acid, were developed. The key labeled intermediate product, 4-guanidinobenzoic acid, was synthesized from cyanamide and 4-aminobenzoic acid in HCl aqueous solution. A convenient method for the synthesis of the β-acyl glucuronide, a common drug metabolite, was developed. The key intermediate product was prepared by the HATU-mediated coupling of 4-[[9-chloro-7-(2,6-difluorophenyl)-5*H*-pyrimido[5,4-d][2]benzazepin-2-yl]amino]-benzoic acid with allyl glucuronate. Sodium ring-[^{14}C]-4-[[9-chloro-7-(2,6-difluorophenyl)-5*H*-pyrimido[5,4-*d*][2]benzazepin-2-yl]amino]-benzoate was prepared from [^{14}C]-cyanamide in two steps in an overall yield of 7%. Sodium carboxyl-[^{14}C]-4-[[9-chloro-7-(2,6-difluorophenyl)-5*H*-pyrimido-[5,4-*d*][2]benzazepin-2-yl]amino]-benzoate was synthesized from carboxyl-[^{14}C]-4-guanidinobenzoic acid in one step in a yield of 35%. [D_4,^{15}N]-4-[[9-chloro-7-(2,6-difluorophenyl)-5*H*-pyrimido[5,4-*d*][2]benzazepin-2-yl]amino]-benzoic acid was synthesized from [$^{15}N_2$]-cyanamide and [D_4]-4-aminobenzoic acid in two steps in an overall yield of 37%. β-Acyl glucuronide of 4-[[9-chloro-7-(2,6-difluorophenyl)-5*H*-pyrimido[5,4-*d*][2]benzazepin-2-yl]amino]-benzoic acid was synthesized from *D*-glucuronic acid in three steps.

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